Hsp90 binds to and promotes the clearance of tau, which is thought to reduce the formation of neurotoxic aggregates. Tau is an intrinsically disordered protein and it is unclear what role, if any, Hsp90 has in controlling its structure and dynamics. Hsp90 cooperates with numerous co-chaperones such as the immunophilin FKBP51, which assists in regulating the folding and processing of client proteins like tau. Defining the precise interactions between tau and the Hsp90 chaperone network is important for understanding the role of tau in Alzheimer's Disease. In this study, nuclear magnetic resonance (NMR) spectroscopy was used to probe the interaction between 15N-labeled tau, Hsp90 and FKBP51. The results demonstrate that two hydrophobic hexapeptide motifs located at residues 275-280 and 306-311 in tau's C-terminus bind to Hsp90 and FKBP51. This was determined by observing a significant reduction in the intensity ratios of HSQC spectra for free tau and tau in complex with Hsp90 and FKBP51. Resonances that show reduced intensities in the absence of line broadening are probably undergoing chemical exchange with a bound conformation. Several residues near the N-terminus of the protein also show a similar reduction in intensity upon addition of Hsp90 and FKBP51. Formation of the ternary complex around the client protein tau is congruent with currently proposed models suggesting that the binding of FKBP51 and Hsp90 assist in tau regulation, thereby triggering its recycling back to the MT surface.

1988-Pos Board B7

Single Molecule AFM Force Spectroscopy Analysis of Alpha-Synuclein Misfolding

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Protein misfolding is a transient state during self-assembly into aggregates defining the molecular mechanism of the development of Alzheimer's, Parkinson's and other neurodegenerative diseases. Misfolding and aggregation of alpha-synuclein (α-Syn) is tightly linked to the development of Parkinson's disease. Here we applied single molecule AFM force spectroscopy (SMFS) to probe transient misfolded states of α-Syn measuring pair-wise interactions between individual α-Syn molecules at conditions that induce conformational transitions associated with enhanced aggregation. In the SMFS approach we probed the interactions between α-Syn covalently attached to the AFM probe and substrate by the C-terminal cysteine. We show that at conditions close to physiological, addition of spermidine results in dramatic increase of the protein's propensity to misfold. Additionally, using SMFS we detected and characterized misfolded dimers of α-Syn, the simplest aggregated form of α-Syn. Our results demonstrate that more than one segment within the protein molecule is responsible for the initial association of α-Syn into dimers and potentially into higher-order oligomers and fibrils. This finding suggests that even the first step of α-Syn self-assembly (dimerization) possesses a certain degree of heterogeneity. We hypothesize that these different misfolded conformations can lead to different types of oligomers and define the aggregation pathway. The marked differences in the misfolding patterns between WT α-Syn and single point mutants might be responsible for the higher propensity of the mutants to aggregate and cause early-onset PD.

The work was supported by grants to YLL from National Institutes of Health (1P01GM091743-01A1 and 1 R01 GM096039-01A1), U.S. Department of Energy Grant DE-FG02-08ER64579, National Science Foundation (EPS - 1004094) and the Nebraska Research Initiative.

1989-Pos Board B8

Macromolecular Crowding Stabilizes the Functional, Non-Toxic State of IAPP by Suppressing its Fibrillation

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The interior of the biological cell is known to be a crowded milieu, which significantly influences protein association and aggregation. As several cell degenerative diseases, such as Parkinsons disease or type-2 diabetes mellitus, are related to the misfolding, self-association and subsequent fibrillation of amyloidogenic peptides, understanding of the impact of macromolecular crowding on these processes is of high biomedical importance. This study focuses on the properties of human islet amyloid polypeptide (hIAPP) in crowded environments of two different kinds: network-like structures formed by polysaccharides and high concentrations of inert globular proteins. Two distinct processes could be distinguished in these crowded solutions: The formation of stable globular off-pathway species, and the usual hIAPP aggregation pathway from a disordered monomeric structure via nuclei formation to fibril formation. To which extent the different pathways are populated is shown to depend markedly on the crowder concentration and the geometry of the confinement. Different to other amyloidogenic peptides, the latter process is retarded or even inhibited at high crowding concentrations, but unchanged on the mechanistic level. As hIAPP is related to type-2 diabetes mellitus and presumably responsible for the disease accompanying β-cell-membrane permeabilization and the final β-cell loss, hIAPP specific cytotoxicity assays were conducted as well. Conversely to the high cytotoxicity exhibited by the normal fibrillation pathway, the data reveal a non-toxic effect for the off-pathway species stabilized through the crowding agents. From these results it can be postulated that cellular crowding is able to stabilize the native, non-toxic and functional conformation of hIAPP inside the biological cell.

1990-Pos Board B9

Surfactant Properties and Interface Induced Aggregation of Tau Proteins

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Abnormal aggregation of microtubule associating protein, tau, into neurofibrillar aggregates , due to protein mutations, is a defining hallmark of several neurological diseases. Recent research indicates that the polymerization of soluble tau proteins into paired helical filaments may be influenced by the hydrophobic properties of its monomers, the presence of inducers and the local environment. In this work, we will discuss our results from using five HTau 40 protein variations: wild type (WT), pseudophosphorylated (7-phos), mutations on the binding domain (P301L), assembly incompetent protein (I277/308P), and mutations on the N terminal (R5L), to study template induced adsorption and aggregation of Tau proteins at a model hydrophobic interface.

Traditional biophysical techniques such as surface pressure vs. time (adsorption isotherms) are used to record adsorption kinetics. We find that even though tau is a soluble protein, it is highly surface active at nanomolar concentrations and demonstrate a two-step adsorption to the hydrophobic interface. Further, the adsorption kinetics is dependent both on the concentration and protein mutation. However, almost all the proteins studied here demonstrate a saturation concentration of a few hundred nanomoles, which is much lower than the bulk concentration where protein aggregation is recorded. Using an active microrheology technique unique to our lab, we also find that surface viscosity of the adsorbed protein films increase by orders of magnitude with time, indicating protein-protein interactions. However, the kinetics of this increase depends on the mutations on the protein. Further, TEM images of the protein solution obtained from the surface indicate the formation of protein oligomers. In summary, our results indicate that the soluble Tau proteins have interesting surfactant properties even at nanomolar concentrations that may play a role in their aggregation during Alzheimer's disease.

1991-Pos Board B10

Coarse Grain Simulations Providing a Unifying Framework for Explaining Polyglutamine Aggregation Mechanism

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Experiments and atomistic simulations show that homopolymeric polyglutamine forms heterogeneous distributions of collapsed, globular conformations in aqueous solutions. Atomistic simulations of monomer-dimer equilibria show that disordered polyglutamine globules associate to form disordered dimers, characterized by interactions between surface residues (the docked state) and interpenetrating chain molecules (the entangled state). Suppression of conformational fluctuations destabilizes the entangled state and inhibits dimerization. Similarly, naturally occurring flanking sequences from huntingtin destabilize the entangled state vis-à -vis the docked state.

Our coarse-grained simulations help to understand the impact of the relative and absolute stabilities of entangled and docked states on the aggregation processes. A phenomenological pair potential is used to model the interplay between these states. Results from our coarse-grained Langevin dynamics simulations are summarized as follows: We define pairwise energy scale ΔU as (Ue - Ud) representing the energy gap between the entangled and docked states, reference state being the bistable situation of ΔU = 0 with Ud = Ue = 4kT, describing the association of homopolymeric polyglutamine molecules. Fixing Ue and increasing ΔU by increasing docked state stability, leads to an increase in the rate of monomer loss and formation of small number of large disordered clusters vis-à -vis the reference bistable state, describing modulation effects of the N-terminal flanking sequence from huntingtin. Conversely, increasing ΔU by destabilizing the entangled state decreases the rate of monomer loss vis-a-vis the reference bistable state accompanied by the formation of large, ordered clusters, describing the effects of C-terminal

flanking sequences from huntingtin. Also, we show that electrostatic repulsions due to these residues retard the rate of monomer loss and large, linear, ordered clusters are formed. Our observations provide a unifying framework, capturing all known features of the early stages of aggregation in polyglutamine containing systems.

1992-Pos Board B11

A Class of Self-Assembling Aliphatic Ultrasmall Peptides as a Model System for Understanding and Preventing Amyloidosis

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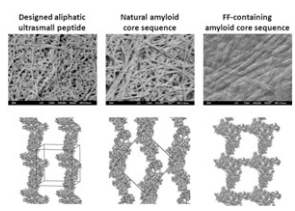
Core sequences of 4-7 residues that form amyloid fibrils have been identified within natural amyloid proteins. However, the mechanism of amyloid aggregation remains unclear. We designed a new class of aliphatic peptides (with 3-6 residues) that self-assemble in water to amyloid β-type fibers via α-helical intermediates. We compared the self-assembly of our designed peptides with core sequences in Amyloid-beta, Amylin and Calcitonin using a multimodal approach. A common feature was the appearance of α-helical intermediates before the final β-turn structures. Another amyloid-beta core sequence containing the diphenylalanine motif was chosen to evaluate the role of aromatic residues in self-assembly. The repeated occurrence of aromatic residues in core sequences has led to widespread conclusions about their key role in driving self-assembly. Surprisingly, the diphenylalanine-containing sequence did not form cross-β aggregates or involve the α-helical intermediate step. Our study puts forth a new, simplified model system to study amyloidosis and indicates that aromatic interactions are not as important as previously postulated. The results provide valuable insight into the early intermediates and factors driving self-assembly, which is necessary for developing small molecule therapeutic drugs that prevent amyloidosis.



1993-Pos Board B12

Discrete Molecular Dynamics Study of Oligomer Formation by N-Terminally Truncated Amyloid B-Protein

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Alzheimer's disease (AD) is strongly linked to amyloid b-protein (Ab). Two predominant alloforms, Ab1−40 and in particular Ab1−42, are known to form toxic oligomers. The N-terminally truncated, pyroglutamated forms of Ab1−40 and Ab1−42 are highly resistant to peptidase degradation and can seed Aβ aggregation. Discrete molecular dynamics (DMD) simulations previously captured in vitro derived distinct Ab1−40 and Ab1−42 oligomer size distributions and predicted that the more toxic Ab1−42 oligomers had more flexible and solvent exposed N-termini than Ab1−40 oligomers. Here, oligomer formation by four N-terminally truncated Ab peptides: Ab3−40, Ab3−42, Ab11−40, and Ab11−42 was examined by the DMD approach. In our simulations, the four N-terminally truncated peptides showed increased oligomerization propensity, consistent with their in vitro tendency to seed aggregation. Conformations formed by Ab11−40 had the lowest b-strand and the highest turn content. The tertiary and quaternary structure of Ab3−4X oligomers was distinctly different from that of Ab11−4X oligomers. Ab3−4X oligomers were characterized by more disordered and solvent exposed N-termini than oligomers formed by the full-length peptides. In contrast, in comparison to Ab1−4X, Ab11−4X oligomers had a more compact structure, facilitated by Val12, resulting in less flexible and less solvent exposed N-termini, suggesting reduced Ab11−4X-mediated toxicity. This unique behavior of the N-termini in Ab peptides might provide a plausible explanation for the experimentally observed increased toxicity of Ab3−4X peptides and their pyroglutamated forms.

1994-Pos Board B13

Intrinsic Disorder and Chaperon-Like Activity of Different Caseins

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Casein is the best characterized milk protein and constitutes over 70-80% of total bovine milk protein. In milk, casein exists as large micelle-like particles that comprise four unrelated proteins (αs1-, αs2-, β-, and κ-casein) and calcium phosphate. Although αs1-, αs2-, β-, and κ-casein present important structural differences, all of them adopt extremely open and flexible conformations, enough to be defined intrinsically disordered proteins (IDPs). Caseins are able to inhibiting protein aggregation and amyloid fibrils formation and this chaperon-like activity could be largely due to their structural disorder. In the present study we discuss the meaning of ''disorder'' in the case of three caseins α, β and k that have similar unordered structure and different sequence. We correlate the different type and disorder degree to the capability of preventing protein aggregation and amyloid formation. The physical-chemical parameters of α, β and k caseins were compared to those of intrinsically unfolded and ideally globular proteins. Moreover, caseins sequences were analyzed by several publicly available disorder-oriented predictors two metaservers, MeDor and meta-PRDOS, and by a neural network algorithm (PONDR). We observed that α, β and k caseins have different degree and type of disorder, depending on the parameters under analysis and criteria used by the different predictors. These data were correlated to experimental results (ThT fluorescence, CD) on the caseins effect on 1-40 β-amyloid peptide fibrillogenesis. Experiments showed that k-casein forms ordered aggregates and that it is able to significantly increase lag-time and reduce fibril amount in Aβ amyloid formation. Our results contribute to clear the role of intrinsically disordered proteins and their mechanism of action by functional order/disorder transitions, and offer insight in the field of prevention and therapy in Alzheimer diseases, and, in general, of amyloid pathologies.

1995-Pos Board B14

Cellular Polyamines Promote Amyloid-Beta Peptide Fibrillation and Modulate the Aggregation Pathways

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The cellular polyamines spermine, spermidine, and their metabolic precursor putrescine, have long been associated with cell-growth, tumor-related gene regulations, and Alzheimer's disease. Here, we show by in-vitro spectroscopy and AFM imaging, that these molecules promote aggregation of amyloid-beta (Aβ) peptides into fibrils and modulate the aggregation pathways. NMR measurements showed that the three polyamines share a similar binding mode to monomeric Aβ(1-40) peptide. Kinetic ThT studies showed that already very low polyamine concentrations promote amyloid formation: addition of 10 μM spermine (normal intracellular concentration is ~1 mM) significantly decreased the lag and transition times of the aggregation process. Spermidine and putrescine additions yielded similar but weaker effects. CD measurements demonstrated that the three polyamines induce different aggregation pathways, involving different forms of induced secondary structure. This is supported by AFM images showing that the three polyamines induce Aβ(1-40) aggregates with different morphologies. The results reinforce the notion that modulation of the Aβ peptide aggregation pathways towards minimally toxic ones by addition of suitable ligands may be a possible therapeutic strategy for Alzheimer's disease.

1996-Pos Board B15

Cyclic N Terminal Fragment of Amylin Forms Non Amyloid Fibers: Implications for Intra- and Inter-Molecular Interactions in Amylin

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Islet amyloid polypeptide (IAPP), also known as amylin, is a 37-residue intrinsically disordered hormone peptide that is secreted together with insulin by the beta cells of the pancreas, and is involved in glucose regulation and gastric emptying. IAPP is implicated in the pathogenesis of diabetes type II, due to its deposition in the form of amyloid fibers in the beta cells of the pancreas, where insulin is produced. IAPP contains a highly conserved, functional disulfide bond that confers a short ring-like structure (N_loop) to the N-terminus of the peptide. Removal of this functional element alters both the mass per length distributions of hIAPP fibers and the kinetics of fibril formation. The mechanism by which the N_loop affects hIAPP aggregation is not yet understood,